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Postharvest biology, quality and shelf life of buckwheat microgreens

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ABSTRACT

Buckwheat microgreens have short shelf life which limits their commercial use. The effects of storage temperature, modified atmosphere packaging (MAP), and wash conditions on quality and shelf life of buckwheat microgreens were assessed. Temperature significantly (P < 0.0001) affected package atmospheres and product quality. At the end of storage, microgreens stored at 1, 5 and 10 °C had smaller microbial populations and less tissue electrolyte leakage than those stored at 15, and 20 °C. Package film oxygen transmission rate (OTR) significantly (P < 0.05) affected package atmospheres. However, differences in quality and shelf life of microgreens packaged in different OTR films were slight and not evident until day 21 of storage. On day 21, buckwheat microgreens packaged in 16.5 pmol/(m2 s Pa) oxygen transmission rate package films were observed to have the freshest appearance with lowest tissue electrolyte leakage. Chlorine (100 mg/L) wash significantly (P < 0.05) reduced microbial populations, initially; however, after 7 days of storage, all washed microgreens experienced accelerated microbial populations. Our findings suggest that buckwheat microgreens should be stored at 5 °C with moderately high O2 (14.0–16.5 kPa) and moderately low CO2 (1.0–1.5 kPa) content to maintain optimal quality and maximal shelf life.

1. Introduction

Microgreens are young and tender cotyledonary leafy greens that are found in a pleasing palette of colors, textures and flavors. They may be used to enhance salads, or as edible garnishes to embellish a wide variety of other dishes. Microgreens, in general, contain higher concentrations of bioactive compounds such as vitamins, minerals, and antioxidants than mature greens (Janovská, Stocková, & Stehno, 2010; Treadwell, Hochmuth, Landrum, & Laughlin, 2010, pp. 1–3; Xiao, Lester, Luo, & Wang, 2012). Buckwheat microgreens specifically are high in antioxidants, flavonoids, carotenoids, and α-tocopherol contents (Janovská et al., 2010). However, like all microgreens, buckwheat microgreens have only a few days of shelf life (Chandra, Kim, & Kim, 2012).

The two most important storage parameters for postharvest shelf life are storage temperature and atmospheric composition (Hodges & Toivonen, 2008). Numerous studies on postharvest shelf life of produce emphasize the importance of temperature control (Brecht, 1995; Watada, Ko, & Minott, 1996), which is generally regarded as the most critical factor in prolonging shelf life of fresh-cut produce (Deza-Durand & Petersen, 2011). However, no information could be found on the optimal commercial storage temperature for buckwheat microgreens.

Modified atmosphere packaging (MAP) effectively prolongs the shelf life of fruits and vegetables by decreasing oxygen (O2) and increasing carbon dioxide (CO2) partial pressures in the package headspace due to the interaction between respiratory O2 uptake and CO2 evolution of the packaged plant tissues, and the selective transfer of gases through packaging films (Kim, Luo, & Gross, 2004). Packaging also reduces contamination of the product by bacteria, mold spores, and other environmental pollutants during storage.

No packaged ready-to-eat microgreens are currently found in supermarkets. Microgreens’ high price and perishability limit them to a specialty market of upscale restaurants and catering establishments. Thus, there is room for expansion of microgreen availability in the market place if temperature and atmospheric conditions favorable to extension of shelf life are determined. Using buckwheat microgreens as a model, we determined the optimal storage conditions and developed protocols for evaluating the...
effect of package film OTR and temperature on the quality of microgreens.

2. Materials and methods

2.1. Sample preparation

Buckwheat (Fagopyrum esculentum Moench CV. Manner) seeds were purchased from Living Whole Foods Inc. (West Springfield, UT, USA), with shell intact. Seeds were soaked in acidified water (pH 5.5–6.0) for 12 h to promote germination according to the supplier’s recommendation. Growing medium, Farfard 3B potting soil consisting of 45/100 g peat moss, 15/100 g vermiculite, 15 g/100 g perlite and 25 g/100 g bark (Griffin Greenhouse & Nursery Supplies, Bridgeton, NJ, USA) was spread evenly in 54 cm × 28 cm × 6 cm trays (vacuum-formed standard 1020 open flats without holes, Growers Supply, Dyersville, IA, USA) containing 2 L water in the bottom. Additional water was sprayed on the surface. The buckwheat seeds were then removed from their acidified water bath and spread evenly over the damp medium, tamping very gently to insure contact with the medium. Seeded trays were kept at 25 °C in the dark for 3–4 days before exposing to light with light irradiance of 9.16 W/m² determined by LI-1000 data logger (LI-COR, Lincoln, NE, USA) for a 12 h photoperiod for an additional 4 days. The trays were sprayed with about 200 mL of water per tray once a day to keep the medium damp. Microgreens were harvested when they reached about 5 cm in height, and the first true leaf was beginning to emerge. The microgreens were cut above the medium line with a pair of sterilized scissors. Damaged microgreens and those with defects were discarded.

2.2. Temperature and package atmosphere treatments

Microgreens used for temperature studies were packaged (10 g/bag) unwashed in sealed 15 cm × 15 cm bags prepared with polyethylene films of 16.6 pmol/(m² s Pa) oxygen transmission rate (OTR). The microgreen samples were stored at 1, 5, 10, 15, or 20 °C for 14 days with evaluations performed on days 0, 3, 6, 10 and 14. Microgreens prepared for package atmosphere treatment were sealed (10 g/bag) unwashed in polyethylene bags (15 cm × 15 cm) with film OTRs of 8.0, 16.6, 21.4 and 29.5 pmol/(m² s Pa) and stored at 5 °C for 21 days with evaluations performed on days 0, 4, 7, 14 and 21.

2.3. Wash conditions

Wash solutions containing 100 mg/L and 50 mg/L free chlorine were prepared with concentrated (6 g/100 g) sodium hypochlorite with pH adjusted to 6.5 using citric acid. A control water wash was prepared without added sodium hypochlorite. Microgreens were placed in mesh bags, then washed in one of the 3 wash solutions with gentle agitation, for 30 s. The microgreens (while in the mesh bags) were centrifuged at 20.5 × g for 200 s with a commercial salad centrifugal dryer (model T-364; Garroute Spin Dryer, Meyer Machine CO, USA) to remove excess water. The washed microgreens, along with unwashed controls (no wash) were packaged in sealed 15 cm × 15 cm bags of 16.6 pmol/(m² s Pa) film OTR. Washed and unwashed packaged microgreens were stored at 5 °C for 21 days and evaluations were performed on days 0, 4, 7, 14 and 21.

2.4. Analysis of packaging headspace atmospheric composition

The CO₂ and O₂ in the headspace of packaged buckwheat microgreens were measured using a gas analyzer (Check mate II, PBI Dansensor Co., Denmark) by inserting the needle of the measuring assembly through a septum adhered to the packaging film.

2.5. Total aerobic mesophilic bacteria

Microgreens (3 g) per replicate were macerated in 27 mL phosphate buffered saline (PBS), using a model 80 Lab stomacher (Seward Medical, London, UK) for 2 min at high speed in filtered stomacher bags. A 50 μL sample of each filtrate or its appropriate dilution was logarithmically spread on agar plates with an automatic spiral plater (Wasp II, Don Whitley Scientific Ltd., West Yorkshire, UK). The total aerobic mesophilic bacteria were plated on tryptic soy agar (TSA, Difco Labs, Sparks, MD, USA) and incubated at 30 °C for 24–48 h. Microbial colonies were counted using an automated colony counter (ProtoCOL SR, Synoptics, Cambridge, UK) and reported as log CFU/g of tissue.

2.6. Tissue electrolyte leakage

Tissue electrolyte leakage was measured following a modified procedure from Kim, Luo, Tao, Saftner, and Gross (2005). Buckwheat microgreens (3 g) were submerged in 150 mL of distilled water for 30 min. The electrical conductivity of the solution was measured using a conductivity meter (model 135A; Orion Research Inc., Beverly City, MA, USA). Total electrolytes of the microgreen samples were determined after freezing at −20 °C for 24 h and thawing at room temperature. Tissue electrolyte leakage was expressed as a percentage of total electrolytes.

2.7. Experimental design and statistical analysis

Package atmospheres, tissue electrolyte leakage, and microbial data were analyzed as two-factor linear models using the PROC MIXED procedure (SAS Institute Inc 1999; Cary, NC). The two factors were storage time and treatment type, which depended on the experiment: storage temperature (1, 5, 10, 15 and 20 °C), package film OTR (8.0, 16.6, 21.4, 29.5 pmol/(m² s Pa)), or wash treatment (no wash, tap water wash, 50 mg/L chlorine, 100 mg/L chlorine) and each had four or five levels. Microbial data were log transformed prior to analysis. Different samples were analyzed on each evaluation day for all studies. Four replications (four bags) per treatment per evaluation period were examined. Assumptions of normality and variance homogeneity of the linear model were checked and the variance grouping technique was used to correct for variance heterogeneity. When effects were statistically significant, means were compared using Sidak adjusted P-values to maintain experiment-wise error ≤0.05.

3. Results and discussion

3.1. Effects of temperature on the quality of buckwheat microgreens

Storage temperature and time significantly (P < 0.0001) affected the O₂ and CO₂ concentrations within the packages (Fig. 1A and B). The O₂ concentration in microgreen packages stored at 1, 5 and 10 °C declined gradually and reached equilibrium on day 10 (around 16 kPa), but in microgreen packages stored at 15 and 20 °C, O₂ concentrations decreased more rapidly from 21 kPa on day 0–8.2 and 6.1 kPa, respectively by day 14. The changes in CO₂ partial pressure followed a reverse trend compared to O₂ (Fig. 1B). The CO₂ concentrations at the different storage temperatures showed similar trends, except at 20 °C which increased sharply at the end of storage.

The initial total aerobic mesophilic bacteria plate count for buckwheat microgreens was 7.2 log which is relatively high, similar
to that of cilantro (Luo, McEvoy, Wachtel, Kim, & Huang, 2004) and baby spinach (Allende, Luo, McEvoy, Artés, & Wang, 2004). The buckwheat microgreens were harvested when less than 5 cm tall and thus had leaves in close proximity to the soil, similar to cilantro and baby spinach. Temperature had significant \( (P < 0.0001) \) effect on total aerobic mesophilic bacteria growth (Fig. 2A). Microgreens stored at 15 and 20 °C had significantly (101 log CFU/g) higher bacterial counts than those stored at 10, 5, and 1 °C after 10 days storage. On day 10 of storage differences among samples stored at 1, 5, and 10 °C were insignificant and likely due to large sample variation. Bacterial populations on buckwheat microgreens stored at 1 °C increased at the end of storage, most likely due to tissue damage resulting from chilling injury.

A slight decline in aerobic mesophilic bacterial populations was seen during the first 3 days of storage for buckwheat microgreens held at 1, 5, and 10 °C, while a slight growth of bacteria was found on samples stored at 15 and 20 °C during the same period. Since mesophilic bacteria generally grow the best at 20–45 °C while psychrotrophic bacteria can grow at 7 °C or less, future studies may need to include both mesophilic and psychrotrophic bacteria in order to capture the full spectrum of bacterial growth in various storage temperature regimes. No literature is available regarding the best storage temperature for buckwheat microgreens. However, the temperature-dependant microbial growth pattern of buckwheat microgreens observed in this study agrees with that of the most perishable leafy greens, e.g. baby spinach, cilantro (Loaiza & Cantwell, 1997; Luo, He, McEvoy, & Conway, 2009).

Tissue electrolyte leakage is an indicator of cell membrane weakening attributable to ripening, or damage caused by physiological stress or mechanical injury (Jiang, Shiina, Nakamura, & Nakahara, 2001). Previously we observed that electrolyte leakage was closely related to the quality and shelf life of fresh-cut produce (Allende et al., 2004; Kim et al., 2004; Luo et al., 2004). Different storage temperatures significantly \( (P < 0.0001) \) affected tissue electrolyte leakage (Fig. 2B). From day 0 to day 3, there was a significant \( (P < 0.05) \) decrease in electrolyte leakage among all treatments. Similar initial decrease in tissue electrolyte leakage was also found on fresh-cut cilantro leaves (Luo et al., 2004). This is very likely due to the initially high influx of electrolytes from ruptured cells during cutting, followed by a recovery, or wound healing process, in which the damaged membranes are shored up during the early stage of cold storage. Storage temperature had no effect on tissue electrolyte leakage during the first 6 days in storage. From day 6 to day 14, microgreens stored at 15 and 20 °C exhibited an accelerated increase in electrolyte leakage while those stored at 5 and 10 °C changed little. Interestingly, microgreens stored at 1 °C had an increase in tissue electrolyte leakage towards the end of storage life, the same time period when package CO\(_2\) content sharply increased (Fig. 1B). Although there was no apparent sign of decay noted in this treatment, these increases in tissue electrolyte leakage and package CO\(_2\) concentration along with increased microbial counts suggest that chilling injury may have occurred when buckwheat microgreens were stored at 1 °C for a prolonged storage period. Chilling injury is
a physiological disorder often found on fruits and vegetables of tropical and subtropical origin when exposed to temperature below 10–15 °C; and some crops of temperate origin can also develop chilling injury when stored at 5 °C or below for a period of time (Bramlage & Meir, 1990). The primary cause of chilling injury is thought to be damage to plant cell membranes. This can then set off a variety of secondary reactions, which may include ethylene production, increased respiration and eventually shortened shelf life (Bramlage & Meir, 1990).

3.2. Effects of package atmosphere on the quality of buckwheat microgreens stored at 5 °C

Package film OTR significantly (\( P < 0.0001 \)) affected the concentration of O2 and CO2 in package headspace (Fig. 3B). Under the tested package configuration (15 cm × 15 cm), product fill weight (10 g) and storage condition (5 °C), O2 and CO2 levels inside 16.6 pmol/(m² s Pa) OTR film bags reached equilibrium of 14.8–15.3 kPa and 1.1–1.4 kPa, respectively, on day 7 and maintained this level until the end of the 14 day storage. The gas composition of the 21.4 and 29.5 pmol/(m² s Pa) OTR film bags showed a similar trend, except equilibrated at higher O2 (16.3–16.8 kPa) and lower CO2 (0.8–1.2 kPa) levels. Similarly, the gas composition of the lowest OTR film (8.0 pmol/(m² s Pa)) maintained the lowest O2 (11.7–13.6 kPa) and highest CO2 (1.5–1.7 kPa) from day 4 until the end of storage.

On day 21, significantly less electrolyte leakage was observed in 16.6 and 29.5 pmol/(m² s Pa) OTR film bags than in 8.0 and 21.4 pmol/(m² s Pa) OTR bags (Fig. 4). Although, microgreens stored in 16.6 pmol/(m² s Pa) OTR films had the best results for package atmosphere, product quality and tissue electrolyte leakage, the similarity between the results for microgreens stored in 16.6 and 29.5 pmol/(m² s Pa) OTR films suggests that a high O2 atmosphere is beneficial for microgreens. Therefore, a perforated film similar to that used for baby spinach may potentially serve as an alternative to OTR films for storage of microgreens and should be tested in future research.

3.3. Effects of washing on the quality of buckwheat microgreens stored at 5 °C

Washed microgreens had significantly (\( P < 0.05 \)) lower aerobic mesophilic bacterial populations than unwashed microgreens on day 0 (Fig. 5A). Water wash, 50 mg/L and 100 mg/L chlorine wash treatments on day 0 reduced aerobic mesophilic bacterial counts on buckwheat microgreens by 0.3 log CFU/g, 0.9 log CFU/g and 1.3 log CFU/g, respectively. Chlorine wash (50 and 100 mg/L) successfully reduced bacterial populations during the first 7 days of storage. During the period from day 7 to day 21, aerobic mesophilic bacterial rebounded by 3.2–3.7 log CFU/g in all wash treatments, especially in water washed microgreens, increasing to 10.3 log CFU/g. This higher growth rate in washed microgreens is probably due to the higher moisture content in the packages, as the result of insufficient drying following the initial wash.

There was no difference in electrolyte leakage among the different treatments during the first 7 days of storage. From day 7 to day 21, electrolyte leakage for all microgreen treatments increased over time (Fig. 5B). Water washed microgreens had the highest electrolyte leakage, followed by 50 mg/L chlorine and 100 mg/L chlorine. After 21 days storage, unwashed microgreens showed the lowest electrolyte leakage, significantly (\( P < 0.05 \)) lower than all of

![Fig. 3. Effect of different package films on changes in partial pressures of O2 (A) and CO2 (B) of buckwheat microgreens. The microgreens were stored at 5 °C for a twenty-one-day period. Data presented are the means of four replications; vertical lines represent standard errors.](image)

![Fig. 4. Effect of different package films on changes in tissue electrolyte leakage of buckwheat microgreens. The microgreens were stored at 5 °C for a twenty-one-day period. Data presented are the means of four replications; vertical lines represent standard errors. Different letters (A–B) indicate significant differences between treatment means at \( \alpha = 0.05 \) on the same day.](image)
the other treatments. These results suggest that wash treatments significantly (P < 0.05) increased electrolyte leakage. This was probably because of high moisture content in packages leading to an increased microbial growth. All washed microgreens had many more water-soaked stems by day 7 than did unwashed microgreens.

4. Conclusions

This study evaluated various postharvest handling conditions on the quality and shelf life of buckwheat microgreens. Results indicate that storage temperature was the most important factor for buckwheat, followed by wash treatment. Storage temperature significantly affected the changes in O₂ and CO₂ composition, tissue electrolyte leakage and microbial growth during storage. The package film oxygen transmission rate significantly affected the package atmosphere composition at equilibrium, and consequently had some effect on product quality and tissue membrane integrity. However, we did not find major differences among package film treatments and no differences until the end of storage. This is because all film treatments provided sufficiently high O₂ and sufficiently low CO₂ to maintain acceptable quality for 21 days at 5 °C. Buckwheat microgreens stored at 5 °C in 16.6 pmol/(m² s Pa) OTR films performed optimally, maintaining highest quality attributes and tissue integrity at the end of the 21 d storage. Washing microgreens with chlorinated solutions resulted in significant reduction in microbial counts on day 0. However, microbial populations rebounded during storage, probably due to excess moisture remaining in packages after washing and drying. Further research is necessary to determine whether by optimizing washing and drying parameters, shelf life can be improved for microgreens that are washed prior to storage.

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